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Response and Remediation Actions Following the Detection of *Naegleria fowleri* in Two Treated Drinking Water Distribution Systems, Louisiana, 2013–2014

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Abstract

Naegleria fowleri causes the usually fatal disease primary amebic meningoencephalitis, typically in people who have been swimming in warm, untreated freshwater. Recently, some cases in the United States were associated with exposure to treated drinking water. In 2013, a case of PAM was reported for the first time in association with exposure to water from a U.S. treated drinking water system colonized with culturable *N. fowleri*. This system and another were found to have multiple areas with undetectable disinfectant residual levels. In response, the water distribution systems were temporarily converted from chloramine disinfection to chlorine to inactivate *N. fowleri* and reduce biofilm in the distribution systems. Once >1.0 mg/L free chlorine residual was attained in all systems for 60 days, water testing was performed; *N. fowleri* was not detected in water samples after the chlorine conversion. This investigation highlights the importance of maintaining adequate residual disinfectant levels in drinking water distribution systems. Water distribution system managers should be knowledgeable about the ecology of their systems, understand potential water quality changes when water temperatures increase, and work to eliminate areas in which biofilm growth may be problematic and affect water quality.

INTRODUCTION

Naegleria fowleri, a thermophilic free-living ameba found naturally in warm freshwater environments such as lakes, ponds, and rivers, causes the disease primary amebic meningoencephalitis (PAM). PAM has a reported mortality of 97% in the United States and occurs when water containing the ameba goes up the nose, crosses the cribriform plate, and enters the brain (Cope & Ali 2016). Surveillance for this infection in the United States has demonstrated that most cases are associated with exposure to warm, untreated freshwater

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while participating in recreational water activities such as swimming or diving (Yoder et al, 2010).

Recent events and research have shown *N. fowleri* to be an opportunistic pathogen living in the biofilms of drinking water distribution systems (Puzon et al., 2009). Long before the first cases of PAM associated with drinking water exposure were identified in the United States, PAM cases with drinking water exposures were reported from regions in Australia in the 1970s and early 1980s (Dorsch et al., 1983). There, drinking water, distributed in overland pipes for hundreds of kilometers, possessed no detectable residual disinfectant by the time it reached the home taps. The successful Australian response to these PAM cases was to implement an ameba monitoring program and boost disinfectant residual levels, which for some systems, required switching to chloramination to maintain disinfectant residual levels over long distances (Robinson & Christy, 1984).

The first cases of PAM in the United States associated with nasal exposure to drinking water were identified in Arizona in 2002 in two children exposed to water from an untreated geothermal well (Marciano-Cabral et al., 2003). More recently, in 2011, the first cases of PAM associated with treated drinking water were identified in two residents of Louisiana who used neti pots containing tap water for sinus irrigation (Yoder et al., 2012). Environmental investigations of the 2011 Louisiana PAM cases included water sampling within the case-patient homes and limited sampling of water from the associated distribution systems; *N. fowleri* was detected only in the homes. In 2013, a case of PAM was reported for the first time in association with exposure to water from a U.S. treated drinking water system colonized with culturable *N. fowleri* (Cope et al., 2015). The case occurred in a 4-year-old child who had played on a lawn water slide supplied with water from two garden hoses connected to the home's outdoor faucet. The home was located in St. Bernard Parish, Louisiana, where one of the neti pot PAM cases occurred in 2011. This finding prompted an environmental investigation of the home and parish water distribution system that ultimately found *N. fowleri* in the water distribution system along with multiple areas of undetectable disinfectant residual (Cope et al., 2015). Here, we describe the response and remediation following the detection of *N. fowleri* in the St. Bernard Parish water distribution system. This response also included a follow-up investigation in DeSoto Parish, where the second 2011 neti pot case-patient resided.

MATERIALS AND METHODS

DeSoto Parish Environmental Investigation

After culturable *N. fowleri* was found in water samples collected from the St. Bernard Parish drinking water distribution system in September 2013 (Cope et al., 2015), health officials conducted an investigation of the DeSoto Parish drinking water system, as both systems were associated with PAM cases in 2011. The DeSoto Parish environmental investigation conducted in September 2013, entailed collection of 10 large-volume ultrafiltration (UF) samples, one water and one sediment sample from a water storage tower serving the 2011 case- patient residence, and the water meter serving the case-patient residence (Figure 1). Field water quality testing was performed in conjunction with water sample collection. Methods used to test these samples, and additional samples collected from the St. Bernard

Parish water system, are described in subsequent sections. Based on the results of the investigation in DeSoto Parish (Table 1) as well as the findings in St. Bernard, health officials decided to perform a chlorine conversion in DeSoto and St. Bernard Parishes.

Chlorine Conversions in St. Bernard and DeSoto Parish Systems

During the St. Bernard Parish case investigation, monochloramine residual, as indicated by measuring total chlorine concentrations, was not detected in 3 of 4 distribution system samples from which *N. fowleri* was cultured (Cope et al, 2015). The St. Bernard and DeSoto Parish water utilities used chloramination to maintain a disinfectant residual in their distribution systems, so water utility and health officials determined that a temporary chlorine conversion was appropriate to inactivate *N. fowleri* and reduce biofilm in the distribution systems. Health officials estimated that a free chlorine level of 1.0 mg/L maintained at all points in the distribution systems and a free chlorine level of 2.0 mg/L maintained in all storage tanks for at least 60 days should inhibit the growth of *N. fowleri*. After notifying the public about the planned chlorine conversion, each water system shut off the ammonia feed and increased the free chlorine feed to initiate the chlorine conversion process. Free chlorine level measurements were conducted weekly at 60– 120 sites in each of the systems to monitor the progress of the chlorine conversion. The sites monitored for chlorine level were selected to be geographically representative taking into consideration population densities and areas with persistently relatively low residual readings (based on historical water quality data from the systems). The 60-day time period started when all sites in each distribution system achieved at least a 1.0 mg/L free chlorine residual. If any site tested fell below 1.0 mg/L, then the 60-day clock was paused until all sites tested were ≥1.0 mg/L.

Sample Collection

Before chlorine conversion, distribution system water and water meter samples were collected from the DeSoto Parish system in September 2013 and water and sediment were collected from a storage tank in December 2013. After the chlorine conversions were completed, sampling of finished and source water began in both DeSoto and St. Bernard Parish systems (Figures 1 & 2). Water samples were collected in the months of January, May, and September 2014 for both systems. Sample sites included service lines, flush or fire hydrants, or other collection points in the distribution system.

Before sample collection, each faucet was purged for 3 minutes to flush out stagnant water. A 1-L grab sample for *N. fowleri* analysis and a 100-mL grab sample for heterotrophic plate count [HPC, Standard Method 9215D (APHA, 2005)] analysis were collected in bottles containing sodium thiosulfate to immediately quench the chlorine residual in a finished water sample (1% w/v ratio). In addition to a grab sample, a large-volume sample was collected by dead-end ultrafiltration (DEUF) (Mull & Hill, 2012; Smith & Hill, 2009) for *N. fowleri* analysis. Between 84 and 776 liters were collected at each site by DEUF, depending on time available and logistical constraints at the sampling locations. The chlorine residual of the water retained in the ultrafilter was immediately quenched by pushing a 1% (w/v) sodium thiosulfate solution through the ultrafilter. Chlorine residual, temperature, pH, turbidity and conductivity were measured in the field. Free and total chlorine residual were

measured using USEPA-approved DPD reagents and HACH Pocket Colorimeter II instrument. Turbidity was measured using a HACH 2100P portable turbidimeter. Temperature, pH, and conductivity were measured using a HACH HQ40d Portable Meter multi-parameter probe.

During storage and transport, samples for ameba testing were maintained at ambient temperature and the HPC sample was kept chilled. Samples were shipped overnight to the Centers for Disease Control and Prevention (CDC) in Atlanta, GA for processing and analysis the following morning.

Naegleria fowleri Detection

Ultrafilters—Ultrafilters were backflushed using a CDC protocol and a backflush solution of WB saline containing 0.1% Tween 80 (Smith & Hill, 2009; Visvesvara & Balamuth, 1975). The recovered backflush solution was centrifuged at 1,500 x *g* for 15 minutes at 25°C and the pelleted concentrate (5–27 mL, median 9 mL) was split three ways: a 750-μL aliquot directly to culture, a 750-μL aliquot extracted for molecular analysis, and the remaining volume subjected to immunomagnetic separation (IMS) (Mull et al., 2013). Following IMS, the resultant sample was split two ways: a 750-μL aliquot directly to culture, and a 750-μL aliquot extracted for molecular analysis.

A stock solution of *E. coli* ATCC 11775 was produced by streaking TSA agar plates and incubating overnight at 37 °C. Plates were flooded with 5–10 mL WB saline and the growth was scraped from each plate using a cell spreader. The suspension was stored in a conical tube at 4 °C. Before the *Naegleria* culture procedure, one hundred microliters of the *E. coli* suspension was spread onto non-nutrient agar (NNA) plates and allowed to absorb in a biosafety cabinet. To the prepared plates, 750 μL of the pre- and post-IMS samples were pipetted directly into the center of the plates and allowed to absorb with minimal agitation to prevent spreading of the inoculum. Plates were incubated at 42°C and examined for the presence of thermophilic ameba trophozoites or cysts for up to 7 days using a standard light microscope at 100 and 200X total magnification. When trophozoites or cysts were observed, the amoebas were harvested in 2 mL WB saline by gently scraping the surface of the agar with a cell scraper. If no amebic forms were observed within 7 days, the entire plate was harvested in 2 mL WB saline. A 750-μL aliquot was extracted for molecular analysis. Nucleic acid was extracted from samples using a universal extraction procedure for environmental samples (Hill et al, 2015) and *N. fowleri* was detected using a species-specific real-time PCR assay (Mull et al, 2013). A direct (pre-culture) sample was considered positive when duplicate real-time PCR crossing threshold (CT) values were ≤42. Confirmation of pre-culture PCR-positive samples was performed by Sanger sequencing using a pair of primers designed to amplify sequences of the 5.8S/ITS rRNA gene. The forward (JNF, GAGATGAACCTGGCGGACC, 1741 to 1759) and reverse (JNR, GGGGTACCCTCTCACATTAGA, 2208–2188) primers were designed to amplify ~468 bp and positions in parenthesis were based on the sequence of *Naegleria fowleri* (GenBank accession no. KT375442). PCR was performed using TaqMan Environmental Master Mix 2.0 (Life Technologies, Grand Island, NY) containing 5 μL of DNA and 0.25 μM of each primer in a 20 μL reaction volume. The PCR amplification conditions were: denaturation at

95 °C for 10 minutes followed by 50 cycles of 95 °C for 15 seconds and 63 °C for 45 seconds. The amplified product was submitted to GeneWiz (South Plainfield, NJ) for Sanger sequencing using the forward primer and the resultant sequences were further trimmed to remove low quality regions at 5' and 3' nucleotides to obtain ~380 bp. Trimmed sequence data were analyzed using a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as *N. fowleri*.

A culture sample was considered positive if amebas were observed on the culture plate and confirmed by duplicate real-time PCR detections.

Grab Samples—Two milliliters of an *E. coli* ATCC 11775 suspension was added to the 1-L grab sample and the entire volume was centrifuged at 1,500 x *g* for 15 minutes at 25°C. The resulting pellet was brought to 2 mL with WB saline and 1-mL each was inoculated onto two prepared NNA plates using the culture methods described for ultrafilter samples. One plate each was incubated at 42 and 44°C and examined for the presence of thermophilic ameba trophozoites or cysts for up to 7 days. However, only samples that showed evidence of thermophilic ameba growth were harvested for PCR confirmation of *N. fowleri*.

Statistical Analyses—Linear regression was performed to evaluate associations between measured physical water quality parameters (temperature, turbidity, conductivity) and HPC. Regression models were stratified by system, included data from all sampling months, and treated each sample independently. Separate models were developed for each water quality parameter. A two-tailed paired t-test was conducted to compare the average total chlorine residuals in the St. Bernard Parish system in May versus September. Statistical analyses were performed in Excel, and p-values ≤ 0.5 were considered statistically significant.

RESULTS

Initial Investigation of DeSoto Parish Water System

Before chlorine conversion, total chlorine residuals in the DeSoto Parish system samples ranged from 0.05–0.44 mg/L (Table 1). *N. fowleri* was detected by culture in 6 of 10 distribution system water samples collected from the DeSoto Parish system. *N. fowleri* was not detected in the 1.1-L water sample or the sediment sample collected from the water storage tank or in biofilm material recovered from the water meter serving the 2011 PAM case patient's home (data not shown).

Chlorine Conversions

Before the chlorine conversion in the St. Bernard Parish system, total chlorine residuals were low and 5 of the 10 distribution samples tested had total chlorine residuals below the detectable level of 0.02 mg/L (Cope et al., 2015). In the St. Bernard system, it took 10 weeks to start the 60-day clock (conversion started September 5, 2013) and 17 weeks to complete the 60-day conversion (November 14, 2013 through January 22, 2014) (Figure 3). For DeSoto, it took 3.5 weeks to start the 60-day clock (conversion started October 8, 2013) and 10 weeks to complete the 60-day conversion (October 30, 2013 through December 29, 2013) (Figure 4). Both systems had to perform extensive unidirectional flushing to achieve the 1.0

mg/L in certain areas, including areas with old cast iron pipe. St. Bernard cleaned storage tanks and reactivated a booster chlorination system at a downstream water storage facility to achieve the 1.0 mg/L at the end of the system. DeSoto used a mobile booster chlorination system that was moved to specific areas to achieve the 1.0 mg/L faster throughout the system.

Distribution System Investigations after Completion of Chlorine Conversions

After the chlorine conversions were completed, free chlorine residuals ranged from 1.0–5.1 mg/L in the St. Bernard system (Table 2) and 1.3–6.5 mg/L in the DeSoto system (Table 3) and over time a decrease in disinfectant residual was observed. In St. Bernard the total chlorine residuals throughout the distribution system were significantly lower in September than in May (P=0.01). Statistical analyses could not be performed for DeSoto due to the switch from measuring free chlorine in January and May to total chlorine in September, but total chlorine residuals in September were generally lower than the free chlorine residuals measured in January.

In the St. Bernard Parish system the pH ranged from 7.1–7.6 (median 7.3) from January to September (data not shown). Turbidity ranged from 0.06–38 NTU (median 0.23 NTU) and conductivity ranged from 310–500 μS/cm (median 380 μS/cm). Temperature increased from January to September, with a median January temperature of 15 °C and a median September temperature of 29 °C. In the DeSoto parish system the pH ranged from 6.1–7.5 (median 6.9) from January to September. Turbidity ranged from 0.27–16 NTU (median 1.1 NTU) and conductivity ranged from 260–440 μS/cm (median 330 μS/cm). Water temperature increased from January to September with a median January temperature of 11 °C and a median September temperature of 26 °C.

There were no significant associations between HPC and measured turbidity or conductivity in either system. However, increasing water temperatures throughout the year were associated with increased HPC concentrations in both parishes. In St. Bernard every 10 °C increase in temperature was associated with a 2.5 $\log_{10}$ increase in HPC concentration (β=0.25, P<0.0001). In DeSoto every 10 °C increase in temperature was associated with a 2.0 $\log_{10}$ increase in HPC concentration (β=0.20, P<0.0001).

No culturable thermophilic amebas were detected in any sample collected in January or May. In September, *N. fowleri* was detected by pre-culture PCR in the large-volume ultrafilter samples from sites B10 and B11 in St. Bernard Parish (Table 3). For both sites, the IMS-treated aliquots and non-IMS-treated aliquots both tested positive. The presence of *N. fowleri* in these samples was confirmed by Sanger sequencing. Sequences were deposited to GenBank under accession numbers MN166032 and MN166031 for samples B10 and B11, respectively.

Thermophilic amebae were cultured from the site B10 sample, but not from site B11. However, these thermophilic amebae tested negative for -*N. fowleri* by PCR. Thermophilic amebae were detected by culture in the large-volume ultrafilter sample from site B9 in St Bernard parish in September, but tested negative for *N. fowleri* by PCR.

DISCUSSION

Following the chlorine conversion, disinfectant residuals in both the St. Bernard and DeSoto distributions systems met or exceeded the 1 mg/L free chlorine target. *N. fowleri* is susceptible to inactivation by chlorine and consequently no *N. fowleri* was detected in either system immediately after the chlorine conversion (Miller et al., 2015). As the year progressed, conditions in the St. Bernard distribution system became favorable to *N. fowleri* growth and persistence. Water temperatures within the distribution system increased, and as a thermophilic organism, *N. fowleri* thrives in warm temperatures (De Jonckheere, 2002). Maintaining the disinfectant residual throughout the distribution system was difficult, as evidenced by a decrease in average concentration over time. Concentrations of HPC also increased throughout the year. HPC is an indicator of bacterial regrowth within a distribution system and is generally considered a good indicator of biofilm growth (Robertson & Brooks, 2003). *N. fowleri* readily attaches to and persists in biofilms (Biyela et al., 2012; Goudot et al., 2012) which increases the disinfectant residual or contact time required for sufficient inactivation (Miller et al., 2015). Thus, an increase in HPC over time is indicative of conditions within the distribution system that are favorable for biofilm growth and persistence and possibly the presence of *N. fowleri*. A study conducted in 1984 found that *N. fowleri* presence in drinking water distribution systems was positively associated with water temperature and colony counts at 35°C (analogous to HPC) and negatively associated with chlorine residual (Esterman et al., 1984). More recent work by Miller et al. showed that a constant free chlorine concentration of >1 mg/L both removed and prevented recolonization of *N. fowleri* in bulk water and biofilm (Miller et al, 2017). Detection of non-viable *N. fowleri* in the St. Bernard system in September points to distribution system conditions that appeared adequate for controlling *N. fowleri*. However, concurrent detection of culturable thermophilic amebas may also be indicative of favorable conditions *for N. fowleri* survival. More research is needed to determine what other factors play a role in the detection of *N. fowleri* in drinking water distribution systems.

In a developed country like the United States, the majority of the population is fortunate to have safe drinking water for which the risk of acquiring enteric illness is low. While treatment of drinking water in the United States has reduced the incidence of waterborne enteric illness since the early 1900s, other infections caused by opportunistic waterborne pathogens are increasingly reported, including *Legionella*, *Pseudomonas* and nontuberculous mycobacteria (Falkinham et al., 2015). *N. fowleri* is also considered an opportunistic waterborne pathogen that can thrive in warm, under-treated, biofilm-rich water distribution systems. Although the number of *N. fowleri* infections reported in the United States is low (0–8 annually), the disease it causes, PAM, has a high mortality and typically affects otherwise young, healthy people. PAM cases, particularly those associated with exposure to drinking water, cause concern and a lack of trust in the safety of the water system among community members.

Because there is no guarantee that even the most vigilant drinking water system can completely eliminate *N. fowleri*, the public should take additional precautions when using tap water for nasal or sinus rinsing. Water used for nasal or sinus rinsing should undergo additional treatment at the point of use, which can include boiling for 1 minute and cooling

before use, filtering using a filter labeled as absolute pore size of 1 micron or smaller, disinfecting with chlorine bleach, or using water that is purchased sterile or distilled.

The focus of this study was not to compare detection methods for *N. fowleri*, but we employed both large volume and small volume detection methods to increase the probability of detecting viable thermophilic amebae in the distribution system. However, it is worth noting that *N. fowleri* was only detected by DEUF sampling. There were no *N. fowleri* detections in corresponding grab samples from each site in the distribution system. This indicates that *N. fowleri* concentration levels were low, and makes the case for large volume sampling for distribution system monitoring for low-level contamination. It is not known whether higher concentrations of *N. fowleri* in water are associated with higher risk of acquiring PAM.

The results of this investigation highlight the importance of maintaining adequate residual disinfectant levels in drinking water distribution systems. Water distribution system managers should be knowledgeable about the ecology of their systems, understand potential water quality changes when water temperatures increase, and work to eliminate areas in which biofilm growth may be problematic and affect water quality.

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REFERENCES

Biyela PT, Hodon R, Brown A, Alum A, Abbaszadegan M, & Rittmann BE 2012 Distribution systems as reservoirs of *Naegleria fowleri* and other amoebae. Journal - American Water Works Association, 104(1), 49–50.

Cope JR, & Ali IK 2016 Primary amebic meningoencephalitis: what have we learned in the last 5 years? Curr Infect Dis Rep, 18(10), 31. [PubMed: 27614893]

Cope JR, Ratard RC, Hill VR, Sokol T, Causey JJ, Yoder JS, Mirani G, Mull B, Mukerjee KA, Narayanan J, Doucet M, Qvarnstrom Y, Poole CN, Akingbola OA, Ritter JM, Xiong Z, Da Silva A, Roellig D, Van Dyke RB, Stern H, Xiao L, & Beach MJ 2015 The first association of a primary amebic meningoencephalitis death with culturable *Naegleria fowleri* in tap water from a US treated public drinking water system. Clinical Infectious Diseases, 60(8), e36–e42. [PubMed: 25595746]

De Jonckheere JF 2002 A century of research on the amoeboflagellate genus *Naegleria*. Acta Protozoologica, 41(4), 309–342.

Dorsch MM, Cameron AS, & Robinson BS 1983 The epidemiology and control of primary amoebic meningoencephalitis with particular reference to South Australia. Trans R Soc Trop Med Hyg, 77(3), 372–377. [PubMed: 6623596]

Esterman A, Roder DM, Cameron AS, Robinson BS, Walters RP, Lake JA, & Christy PE 1984 Determinants of the microbiological characteristics of South Australian swimming pools. Appl Environ Microbiol, 47(2), 325–328. [PubMed: 6424565]

Falkinham JO, Pruden A, & Edwards M 2015 Opportunistic premise plumbing pathogens: increasingly important pathogens in drinking water. Pathogens, 4(2), 373–386. [PubMed: 26066311]

Goudot S, Herbelin P, Mathieu L, Soreau S, Banas S, & Jorand F 2012 Growth dynamic of *Naegleria fowleri* in a microbial freshwater biofilm. Water Research, 46(13), 3958–3966. [PubMed: 22695355]

Hill V, Narayanan J, Gallen R, Ferdinand K, Cromeans T, & Vinje J 2015 Development of a nucleic acid extraction procedure for simultaneous recovery of DNA and RNA from diverse microbes in water. Pathogens, 4, 335–354. [PubMed: 26016775]

Marciano-Cabral F, MacLean R, Mensah A, & LaPat-Polasko L 2003 Identification of *Naegleria fowleri* in domestic water sources by nested PCR. Appl Environ Microbiol, 69(10), 5864–5869. [PubMed: 14532037]

Miller HC, Wylie J, Dejean G, Kaksonen AH, Sutton D, Braun K, & Puzon GJ 2015 Reduced efficiency of chlorine disinfection of *Naegleria fowleri* in a drinking water distribution biofilm. Environmental Science and Technology, 49(18), 11125–11131. [PubMed: 26287820]

Miller HC, Morgan M, Wylie J, Kaksonen A, Sutton D, Braun K, Puzon G 2017 Elimination of *Naegleria fowleri* from bulk water and biofilm in an operational drinking water distribution system. Water Research, 110, 15–26. [PubMed: 27974249]

Mull B, & Hill VR 2012 Recovery of diverse microbes in high turbidity surface water samples using dead-end ultrafiltration. J Microbiol Methods, 91(3), 429–433. [PubMed: 23064261]

Mull BJ, Narayanan J, & Hill VR 2013 Improved method for the detection and quantification of *Naegleria fowleri* in water and sediment using immunomagnetic separation and real-time PCR. J Parasitol Res, 2013: 608367. [PubMed: 24228172]

Puzon G, Lancaster J, Wylie J, & Plumb I 2009 Rapid detection of *Naegleria fowleri* in water distribution pipeline biofilms and drinking water samples. Environmental Science and Technology, 43 (17): 6691–6. [PubMed: 19764236]

Robertson W, & Brooks T 2003 The role of HPC in managing the treatment and distribution of drinking-water In Heterotrophic Plate Counts and Drinking-water Safety C. J. Bartram J, Exner M, Fricker C, Glasmacher A (Ed.),IWA Publishing, London, pp. 233–244.

Robinson B & Christy PE 1984 Disinfection of water for control of amoebae. Water, 21–24.

Smith CM, & Hill VR 2009 Dead-end hollow-fiber ultrafiltration for recovery of diverse microbes from water. Appl Environ Microbiol, 75(16), 5284–5289. [PubMed: 19561183]

Standard methods for the examination of water and wastewater 2005 21st edn American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Visvesvara GS, & Balamuth W 1975 Comparative studies on related free-living and pathogenic amebae with special reference to *Acanthamoeba*. J Protozool, 22(2), 245–256. [PubMed: 807717]

Yoder JS, Eddy BA, Visvesvara GS, Capewell L, & Beach MJ 2010 The epidemiology of primary amoebic meningoencephalitis in the USA, 1962–2008. Epidemiol Infect, 138(7), 968–975. [PubMed: 19845995]

Yoder JS, Straif-Bourgeois S, Roy SL, Moore TA, Visvesvara GS, Ratard RC, Hill VR, Wilson JD, Linscott AJ, Crager R, Kozak NA, Sriram R, Narayanan J, Mull B, Kahler AM, Schneeberger C, da Silva A, Poudel M, Baumgarten KL, Xiao L, Beach MJ 2012 Primary amebic meningoencephalitis deaths associated with sinus irrigation using contaminated tap water. Clin Infect Dis, 55(9), e79–85. [PubMed: 22919000]

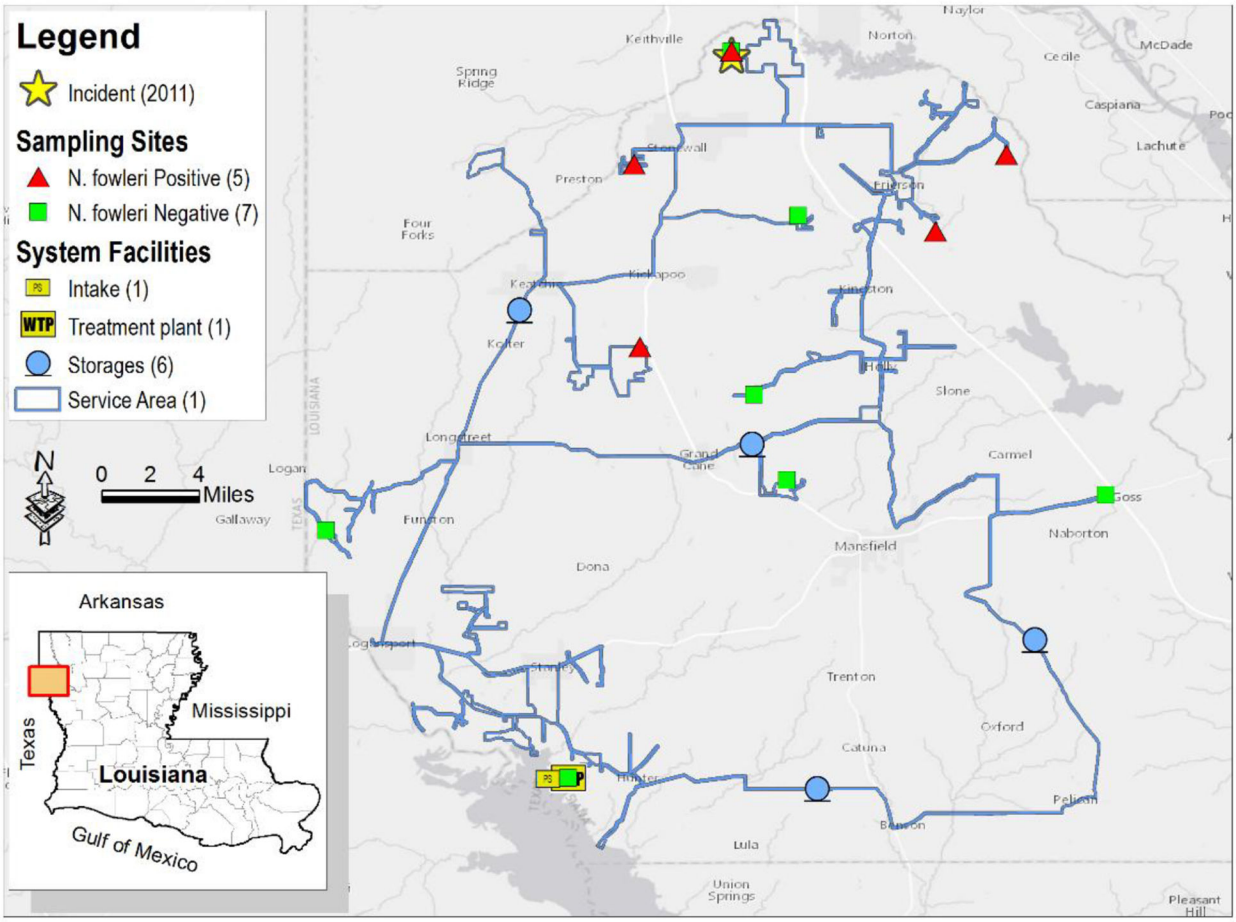

Figure 1.
Map of DeSoto Parish distribution system with locations of sampling sites and PAM case residence.

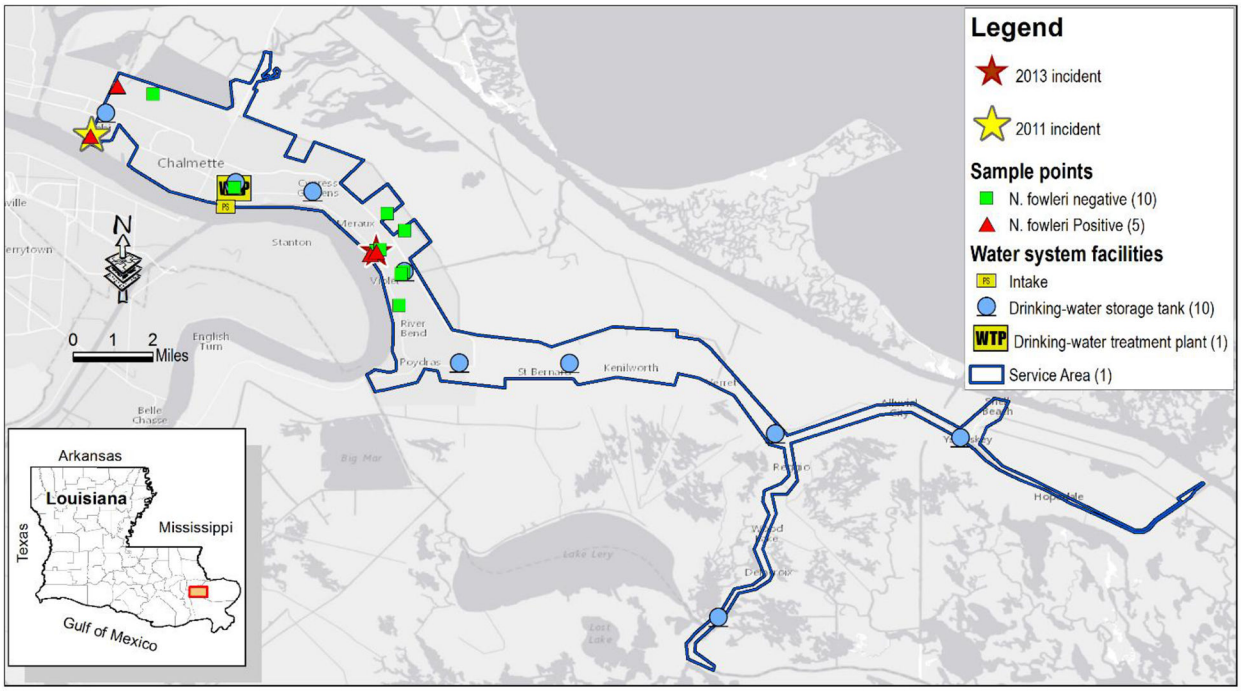

Figure 2.

Map of St. Bernard Parish distribution system with locations of sampling sites and PAM case residences.

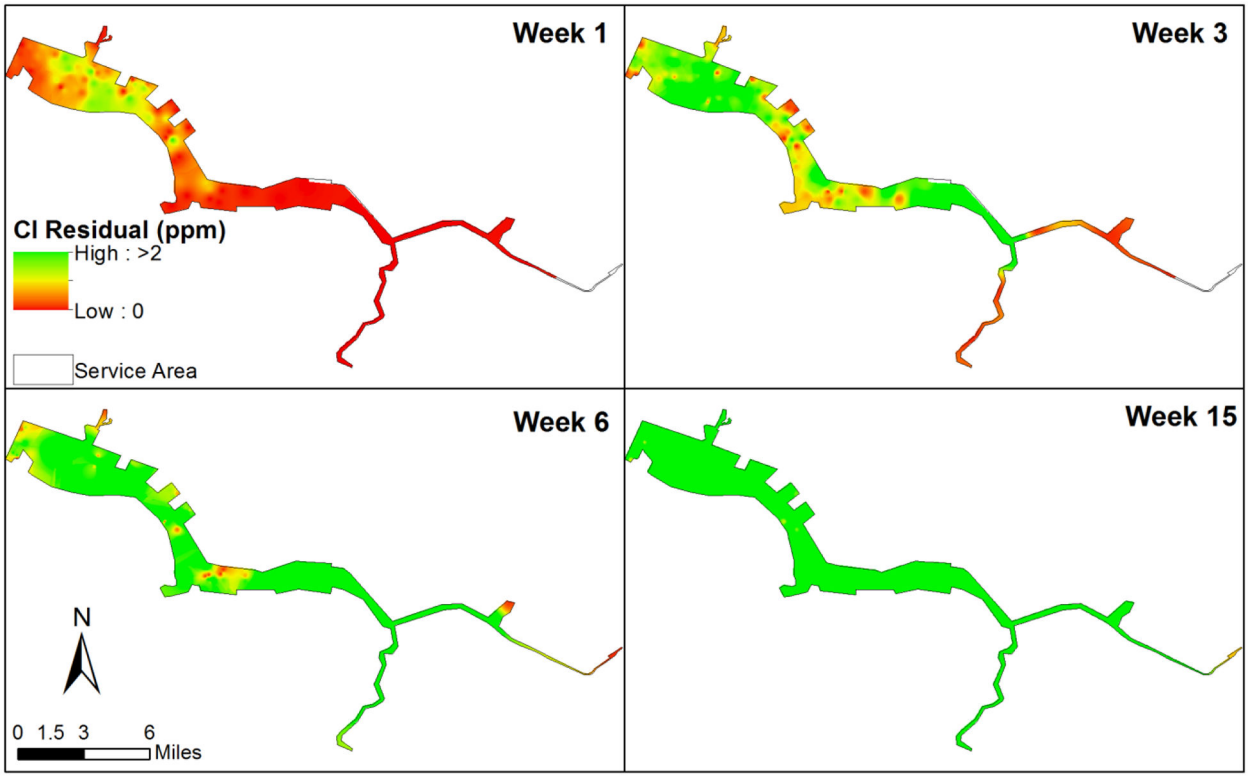

Figure 3.
Progression of chlorine conversion, St. Bernard Parish, 2013–2014.

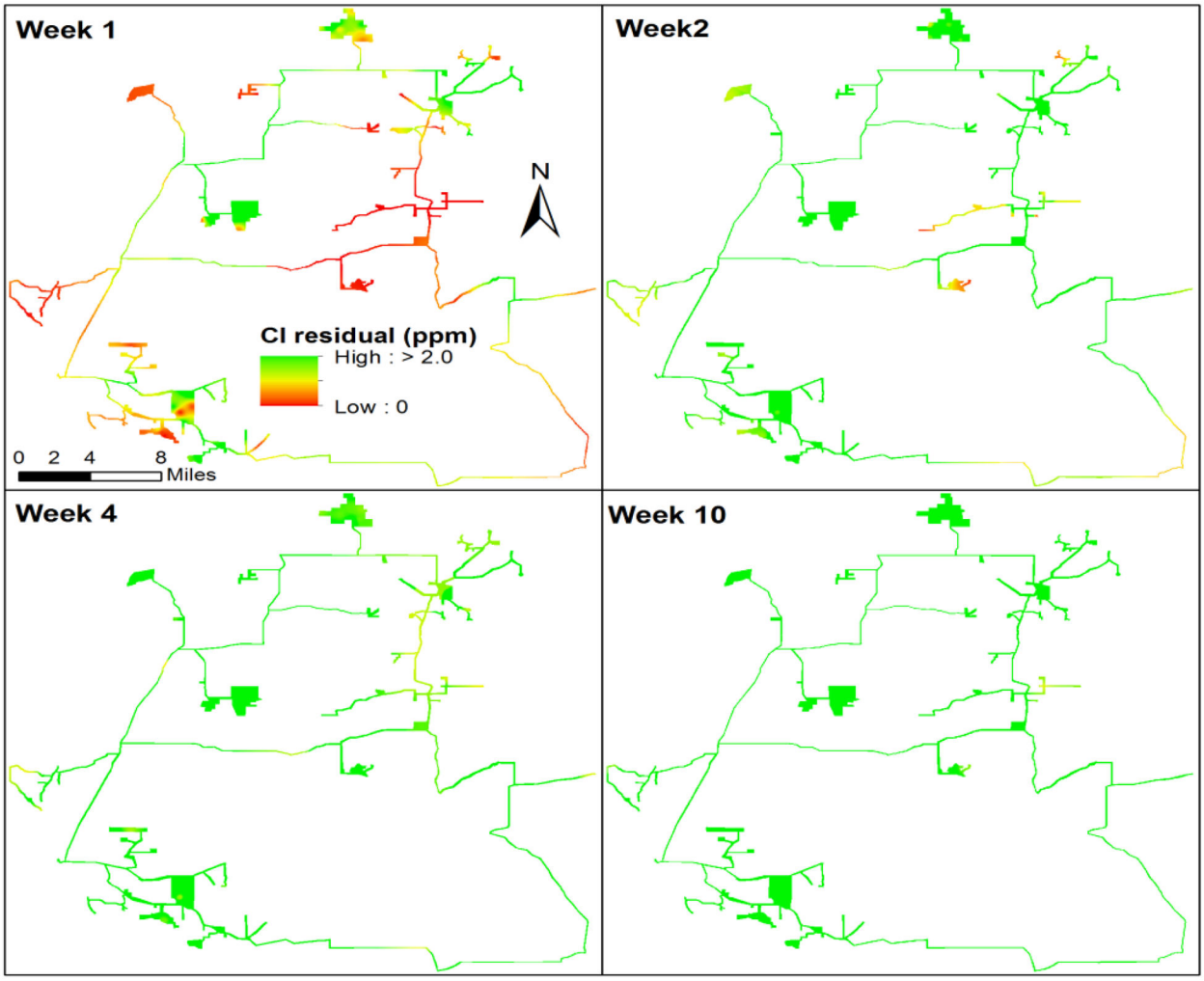

Figure 4.
Progression of chlorine conversion, DeSoto Parish, 2013.

Table 1.

Measured water quality parameters, volume collected for *N. fowleri* testing, and *N. fowleri* detections for water samples collected from DeSoto parish system before chlorine conversion, September 2013.

Site ID	Location	Total chlorine (mg/L)	Temperature (°C)	Volume collected (L)	*N. fowleri* culture
D1	**Point of collection sample tap**	0.10	29	737	+
D2	**Flush hydrant**	0.05	28	775	+
D3	**Flush hydrant**	0.05	30	641	+
D4	**Flush hydrant**	0.08	34	231	+
D5	**Point of collection sample tap**	0.40	26	246	−
D6	**Fire hydrant**	0.20	26	206	−
D7	**Fire hydrant**	0.44	32	418	+
D8	**Flush hydrant**	0.15	31	598	+
D9	**Point of collection sample tap**	0.10	23	105	−
D10	**Fire hydrant**	0.09	30	565	−

Table 2.

Measured water quality parameters, heterotrophic plate count (HPC), and *N. fowleri* detections for DeSoto parish system water samples collected after chlorine conversion, January-September 2014.

Site ID	Location	Month	Temp. (°C)	Free Chlorine (mg/L)	Total Chlorine (mg/L)	HPC (CFU/mL)	Volume collected (L)	*N. fowleri* culture
D1	**Point of collection sample tap**	Jan	12	3.7		<1.0E−02	382	−
		May	23	2.4		1.0E+00	739	−
		Sept	26		0.98	2.8E+01	738	−
D2	**Flush hydrant**	Jan	13	4.1		5.0E−02	285	−
		May	21	0.06		>1.0E+01	776	−
		Sept	25		1.2	1.9E+01	776	−
D3	**Flush hydrant**	Jan	13	2.2		5.0E−02	229	−
		May	23	0.91		1.3E+01	684	−
		Sept	27		1.3	1.6E+01	640	−
D4	**Flush hydrant**	Jan	13	5.4		1.0E−02	201	−
		May	22	1.4		1.0E+00	232	−
		Sept	27		1.3	6.2E+02	231	−
D5	**Point of collection sample tap**	Jan	11	4.2		1.9E−01	300	−
		May	22	0.3		2.8E+00	247	−
		Sept	28		0.90	1.8E+02	246	−
D6	**Fire hydrant**	Jan	10	1.3		2.6E+00	221	−
		May	23	5.5		1.3E+00	218	−
		Sept	26		0.90	5.1E+01	204	−
D7	**Fire hydrant**	Jan	8.8	6.5		8.0E−02	225	−
		May	22	2.9		1.0E−01	416	−
		Sept	26		1.6	4.9E+01	461	−
D8	**Flush hydrant**	Jan	8.9	5.8		<1.0E−02	446	−
		May	21	2.4		7.5E+02	598	−
		Sept	25		0.63	6.0E+01	598	−
D9	**Point of collection sample tap**	Jan	14	3.5		5.0E−02	159	−
		May	22	1.2		3.6E+00	106	−
		Sept	27		1.2	6.1E+02	106	−
D10	**Fire hydrant**	May	21	8.7		<1.0E−01	565	−
		Sept	26		2.8	1.0E+02	573	−
D11	**Flush hydrant**	Jan	10	2.0		<1.0E−02	252	−
D12	**Distribution system point of entry**	Jan	NT	NT	NT	NT		NT
		May	23	4.8		<1.0E−01	106	−
		Sept	26		6.8	<1.0E+00	106	−

NT: not tested

Table 3.

Measured water quality parameters, heterotrophic plate count (HPC), and *N. fowleri* detections for St. Bernard parish water samples collected after chlorine conversion, January-September 2014.

Site ID	Location	Month	Temp. (°C)	Free chlorine (mg/L)	Total chlorine (mg/L)	HPC (CFU/mL)	Volume collected (L)	*N. fowleri* culture
		Jan	13	3.8		<1.0E−02	368	−
B1	**Service line hose bib**	May	26		0.7	>2.0E+02	160	−
		Sept	29		2.2	4.0E+02	159	−
		Jan	13	4.3		5.0E+00	317	−
B2	**Main line**	May	22		3.0	1.5E+02	149	−
		Sept	30		0.4	5.0E+02	148	−
		Jan	13	4.2		<1.0E−02	235	−
B3	**Main line**	May	22		3.2	2.7E+01	117	−
		Sept	28		1.1	1.8E+02	125	−
		Jan	15	2.2		1.5E+01	84.4	−
B4	**Main line**	May	23		3.1	7.6E+00	155	−
		Sept	29		2.4	7.0E+01	138	−
		Jan	13	4.8		<1.0E−02	135	−
B5	**Fire hydrant**	May	22		3.0	9.8E+00	235	−
		Sept	29		2.3	1.1E+02	236	−
		Jan	16	1.1		<1.0E−02	137	−
B6	**Fire hydrant**	May	25		1.4	>2.0E+02	202	−
		Sept	30		0.7	6.0E+02	201	−
		Jan	15	3.3		<1.0E−02	158	−
B7	**Service line**	May	22		3.0	7.0E+01	511	−
		Sept	30		0.5	>2.0E+03	511	−
		Jan	14	2.4		<1.0E−02	102	−
B8	**Service line**	May	23		3.1	2.2E+01	341	−
		Sept	29		1.5	3.3E+02	341	−
		Jan	16	5.1		3.0E+01	327	−
B9	**Service line**	May	23		2.7	>2.0E+02	416	−
		Sept	30		0.1	1.6E+03	450	−[a]
		Jan	16	1.0		3.5E+01	131	−
B10	**Service line**	May	25		1.6	6.0E−01	350	−
		Sept	29		0.4	1.8E+03	356	−[b]
		Jan	16	1.3		<1.0E−02	131	−
B11	**Service line**	May	26		0.5	>2.0E+02	304	−
		Sept	29		0.8	4.6E+02	319	−[c]
		Jan	NT	NT		NT	NT	NT
B12	**Distribution system point of entry**	May	21		4.0	5.9E+00	138	−
		Sept	30		4.2	7.0E+01	100	−

[a] Amebic growth observed on culture plate, but post-culture PCR negative for *N. fowleri*

[b] Pre-culture PCR was positive and amebic growth observed on culture plate, but post-culture PCR negative. *N. fowleri* presence was confirmed by sequencing pre-culture DNA.

[c] Pre-culture PCR was positive, but no amebic growth observed on culture plate. *N. fowleri* presence was confirmed by sequencing pre-culture DNA.

NT: not tested